

H36.D2.B7 ANTI-TISSUE FACTOR LIGHT CHAIN VARIABLE REGION

GACATTCAGATGACCCAGTCTCCTGCCTCCAGTCTGCATCTCTGGGAGAAAGTGTCAACCATCATGC
D I Q M T Q S P A S Q S A S L G E S V T I T C

CTGGCAAGTCAGACCATTGATACATGGTTAGCATGGTATCAGCAGAAACCAGGAAATCTCCTCAGCTC
L A S Q T I D T W L A W Y Q Q K P G K S P Q L

CTGATTATGCTGCCACCAACTTGGCAGATGGGTCCCATCAAGTTTCAGTGGCAGTGGATCTGGCACA
L I Y A A T N L A D G V P S R F S G S G S G T

AAATTTTCTTTCAAGATCAGCAGCCTACAGGCTGAAGATTTTGTAATATT TACTGTCAACAAGTTTAC
K F S F K I S S L Q A E D F V N Y Y C Q Q V Y

AGTTCCTCATTACGTTTCGGTGTGGGACCAAGCTGGAGCTGAAA
S S P F T F G A G T K L E L K

FIG. 1A

H36.D2.B7 ANTI-TISSUE FACTOR HEAVY CHAIN VARIABLE REGION

GAGATCCAGCTGCAGCAGTCTGGACCTGAGCTGGTGAAGCCTGGGGCTTCAGTGCAGGTATCCTGCAAG
E I Q L Q Q S G P E L V K P G A S V Q V S C K

ACTTCTGGTTACTCATTCACTGACTACAAACGTGTACTGGGTGAGGCAGAGCCATGGAAAGAGCCCTTGAG
T S G Y S F T D Y N V Y W V R Q S H G K S L E

TGGATTGGATATATTGATCCCTTACAAATGGTATTACTATCTACGACCAGAACTTCAAGGGCAAGGCCACA
W I G Y I D P Y N G I T I Y D Q N F K G K A T

TTGACTGTGACAAAGTCTTCCACCACAGCCCTTCATGCATCTCAACAGCCCTGACATCTGACGACTCTGCA
L T V D K S S T T A F M H L N S L T S D S A

GTTTATTTCTGTGCAAGAGATGTGACTACGGCCCTTGACTTCTGTTGGGGCCAAAGGCACCACTCTCAGTC
V Y F C A R D V T T A L D F W G Q G T T L T V

TCCTCA
S S

FIG. 1B

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ANTIBODY	APPARENT K_1 , M^{-1}	APPARENT K_1 , M
BY ELISA		
D2	5.2×10^9	1.9×10^{-10}
I47	6.5×10^9	1.5×10^{-10}
K73	9.8×10^9	1.0×10^{-10}
K80	2.3×10^9	4.3×10^{-10}
L102	2.5×10^9	4.0×10^{-10}
L133	1.7×10^9	5.9×10^{-10}
BY BIACore		
H36	3.1×10^{10}	3.2×10^{-11}
I43	2.3×10^9	4.3×10^{-10}
I47	3.2×10^9	3.1×10^{-10}
L133	4.6×10^9	2.2×10^{-10}
M107	1.1×10^9	9.1×10^{-10}

FIG. 2

ANTIBODY NAME	% INHIBITION ANTIBODY PREINCUBATED WITH TF/VIIa
D1	0
D1B	1
H31	4
H36	95
I43	1
J131	7
K80	0
K82	0
K87	1
L97B	7
L101	0
L102	0
L105	0
L133	0
M5	1
M107	34

FIG. 3

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ANTIBODY NAME	<u>% INHIBITION</u> TF PREINCUBATED WITH ANTIBODY PRIOR TO ADDITION OF VIIa	<u>% INHIBITION</u> TF PREINCUBATED WITH VIIa PRIOR TO ADDITION OF ANTIBODY
D1	15	nd
D1B	48	12.7
H31	64	21
H36	0	0
I43	68	55
J131	38	11
K80	12	nd
K82	0	nd
K87	0	nd
L96	0	nd
L101	38	11
L102	14	nd
L105	4	nd
L133	13	nd
M5	0	nd
M107	0	nd

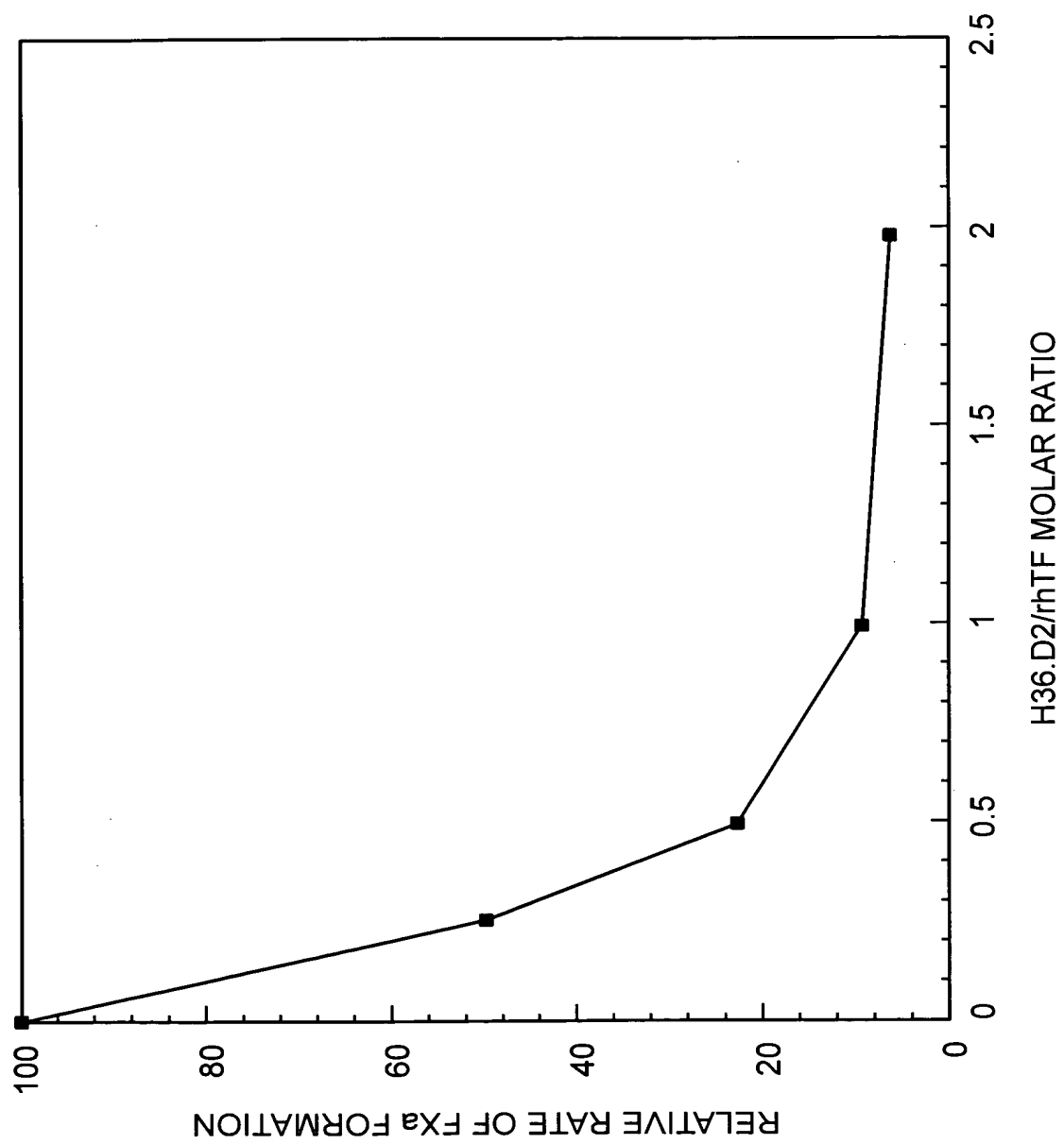
FIG. 4

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[rhTF], nM	[H36.D2], nM	H36.D2/rhTF MOLAR RATIO	CLOTTING TIME (SECONDS)	% INHIBITION OF rhTF FUNCTION
0.0048	0	0	102.3	0
	1.61	335.4	114.3	31.3
	3.23	670.8	121.3	45.8
0.023	0	0	77.6	0
	1.61	70.0	85.3	52.2
	3.23	140.0	91.1	65.2
0.092	0	0	77.6	0
	1.61	70.0	85.3	52.2
	3.23	140.0	91.1	65.2
0.46	0	0	49.3	0
	3.23	35.1	65.8	65.2
	6.45	70.1	88.5	90.2
2.30	0	0	113.3	95.7
	12.90	140.2		
11.52	0	0	32.6	0
	6.45	14.0	52.7	82.4
	12.90	28.0	80.2	96.7
	32.30	70.2	117.9	99.3
	0	0	23.9	0
	16.10	7.0	47.1	94.4
	32.30	14.0	95.2	99.7
	64.50	28.0	115.3	99.9
	0	0	22.2	0
	16.10	1.4	30.2	93.4
	32.30	2.8	46.0	98.8
	64.50	5.6	87.6	99.9
	161.30	14.0	114.0	100.0

FIG. 5

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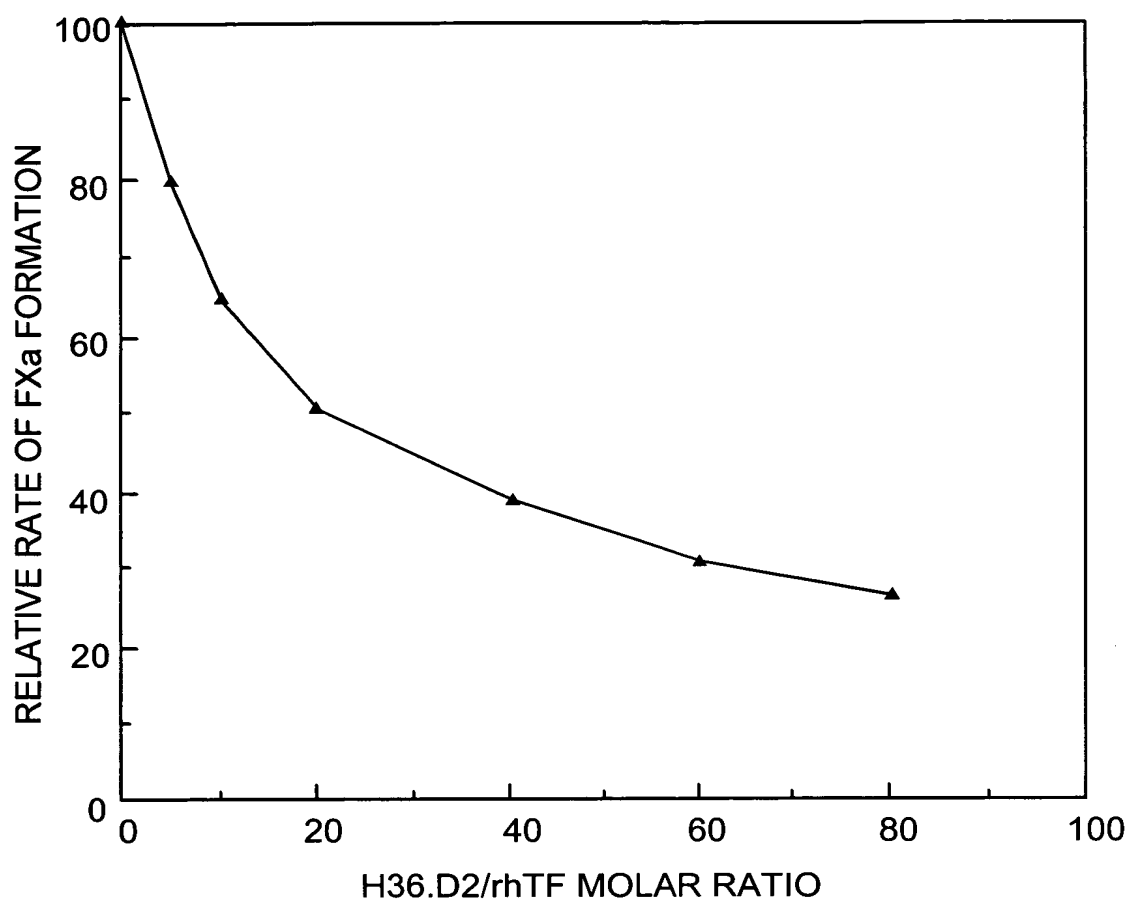


FIG. 6B

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H36.D2 CONCENTRATION (ng)	<u>% INHIBITION</u> CELLS (TF/FVII) AND H36.D2 PREINCUBATED PRIOR TO FX ADDITION	<u>% INHIBITION</u> FX AND H36.D2 ARE ADDED SIMULTANEOUSLY TO CELLS (TF/FVII)
0	0	0
50	88	nd
100	92	nd
200	97	nd
800	nd	76
1600	nd	78
3200	nd	92

FIG. 7

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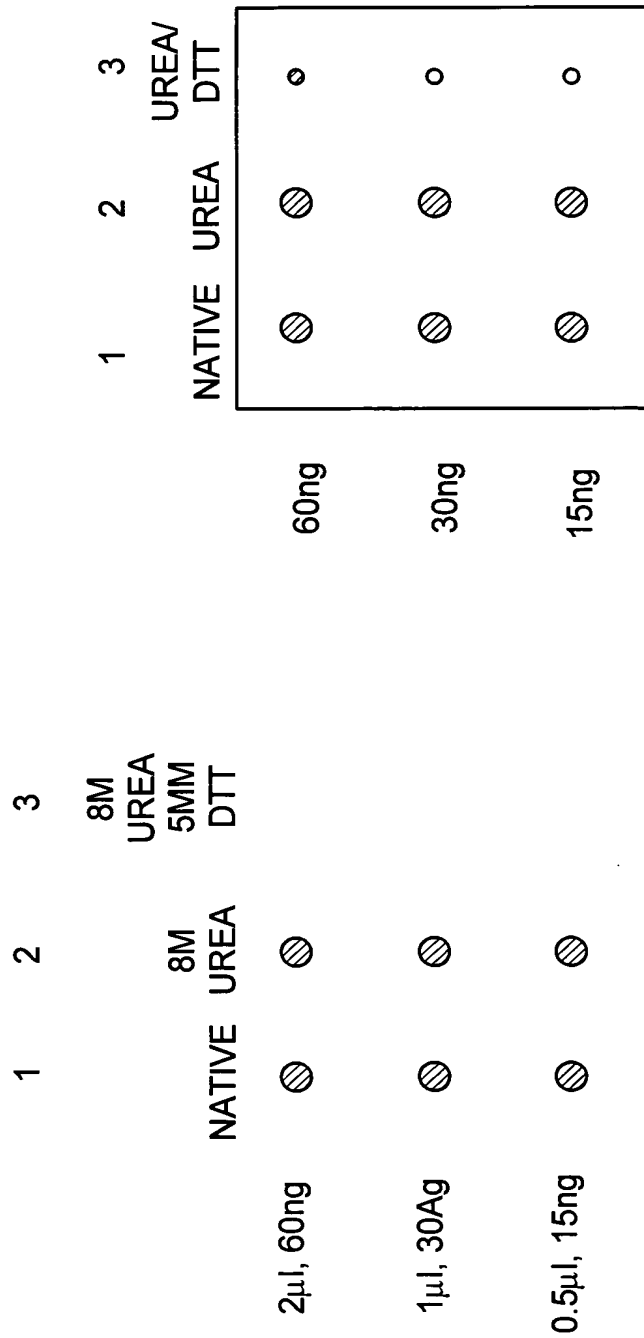


FIG. 8A

FIG. 8B

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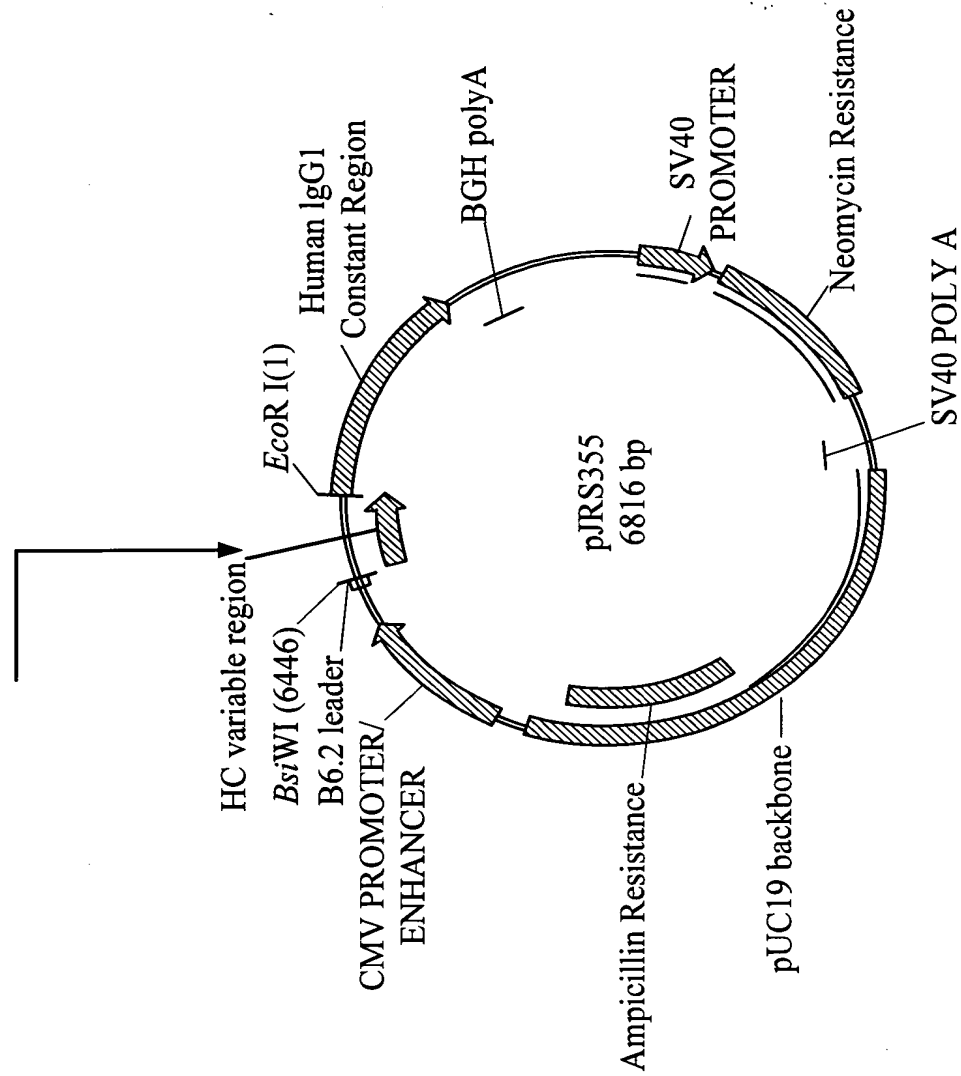


FIG. 9B

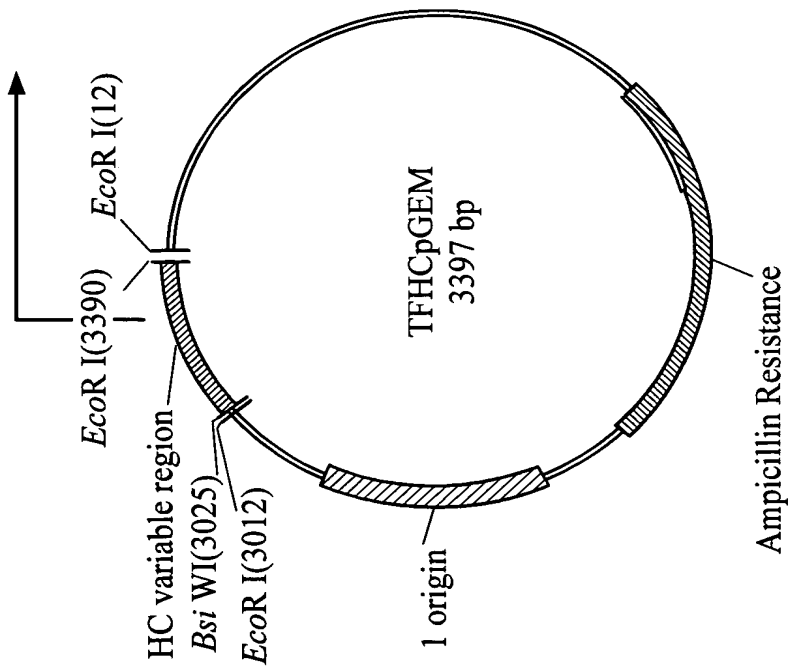


FIG. 9A

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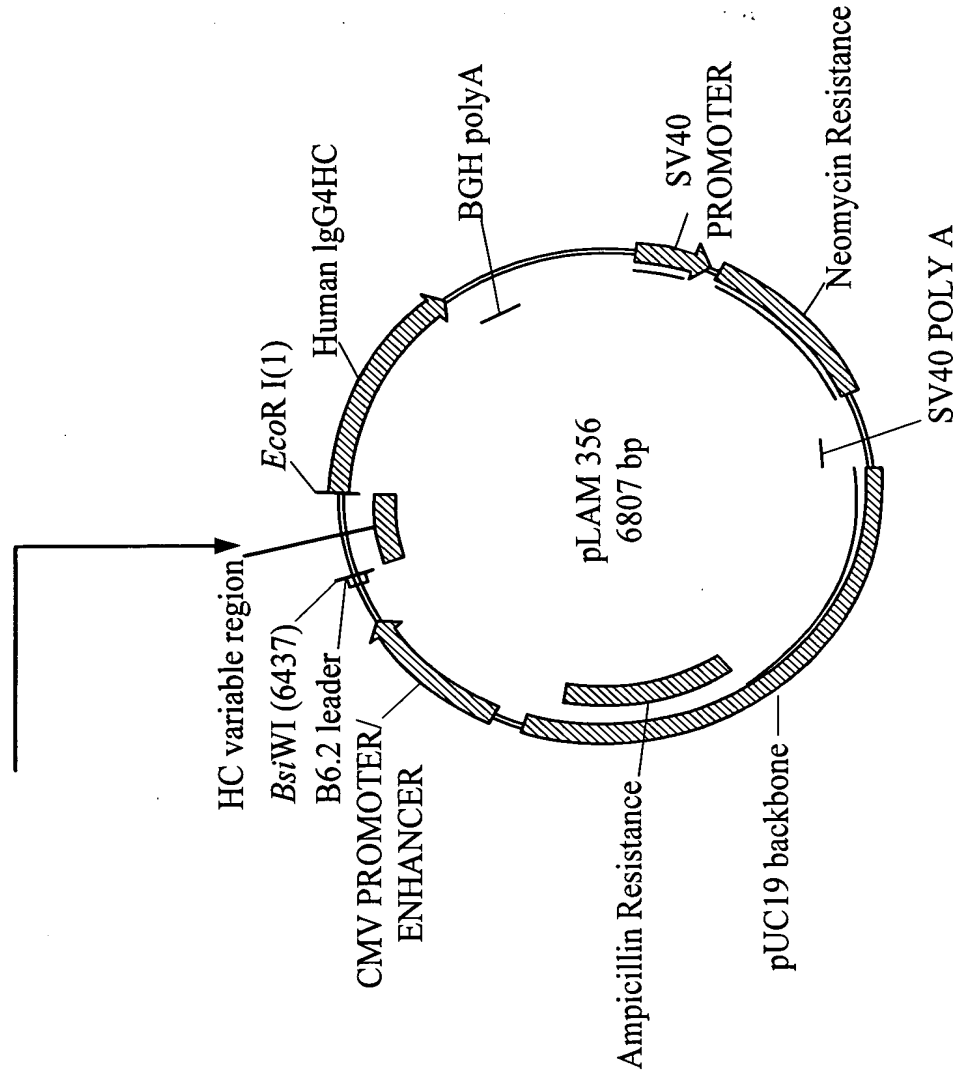


FIG. 9D

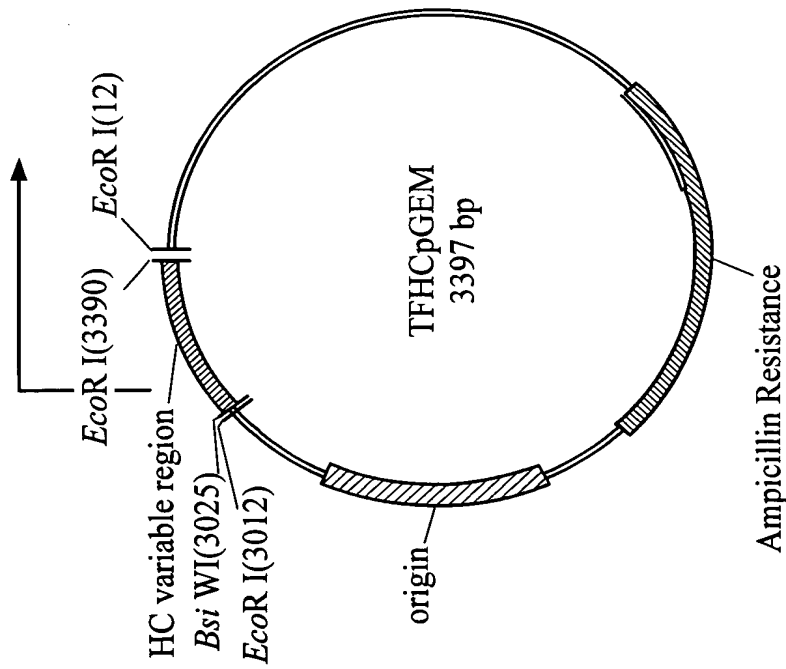


FIG. 9C

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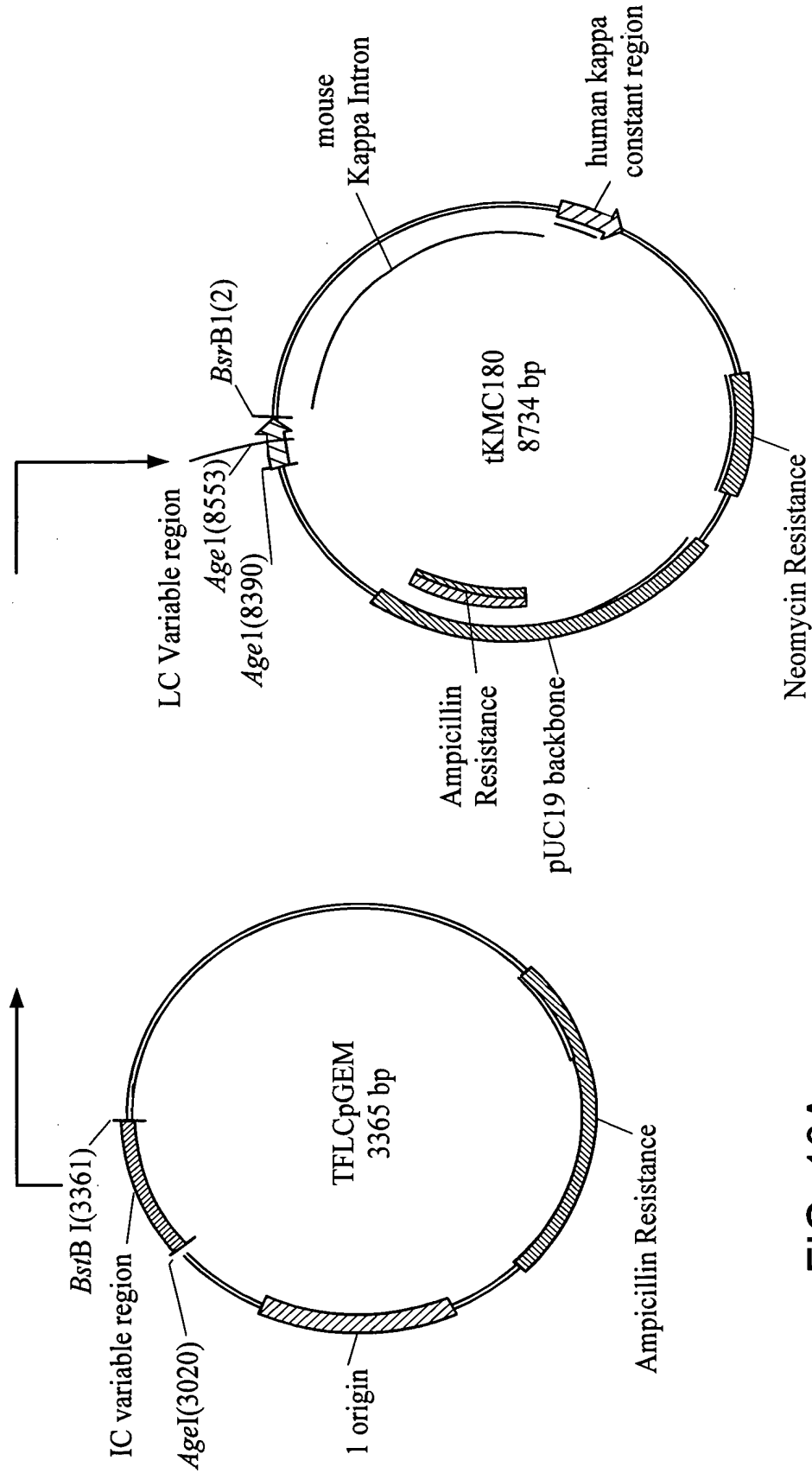


FIG. 10B

FIG. 10A

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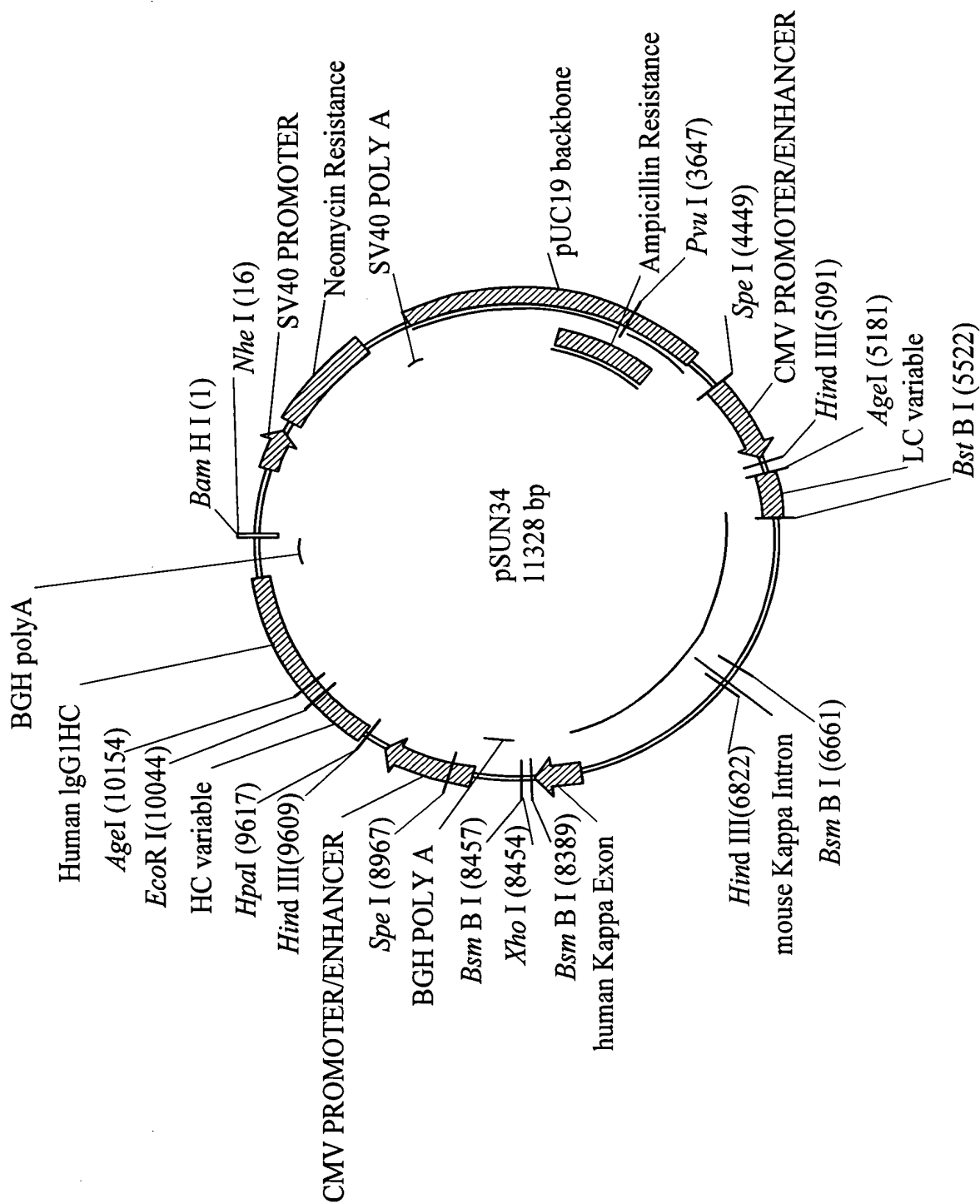


FIG. 11

Humanization of anti-Tissue Factor Antibody cH36

Sequences of Partially and Fully Humanized Light Chain (LC) Variable Regions

Light Chain (LC) FR Sequences

FR1 (23 AA)	FR2 (14 AA)	FR3 (32 AA)	FR4 (10 AA)	Names
1 10 20 35 47 57 60 70 80 86 98 107				
DIQMTQSPASQASLGE SVTITC WYQQKPGKSPQ LIY GVPSRFSGSGSGTKFSFKISSLQAE DFVNYYC FGAGTKLE LK				cH36-LC
DIQMTQSPASQASLGE SVTITC WYQQKPGKSPQ LIY GVPSRFSGSGSGTKFSFKISSLQAE DFVNYYC FGAGTKLE IK				LC-03
DIQMTQSPASQASLGE SVTITC WY L QKPGKSPQ LIY GVPS FSGSGSGTKFSFKISSLQAE DFVNYYC FGAGTKLE IK				LC-04
DIQMTQSPAS L ISASV GDRVTITC WY L QKPGKSPQ LIY GVPSRFSGSGSGTKFSFKISSLQAE DFVNYYC FG Q GTKLE IK				LC-05
DIQMTQSPASQASLGE SVTITC WY L QKPGKSPQ LIY GVPSRFSGSGSGTKFSFKISSLQAE DFVNYYC FG Q GTKLE IK				LC-06
DIQMTQSPASQASLGE SVTITC WY L QKPGKSPQ LIY GVPSRFSGSGSGTKFSFTISSLQ PEDFVNYYC FG Q GTKLE IK				LC-07
DIQMTQSPASQASLGE SVTITC WY L QKPGKSPQ LIY GVPSRFSGSGSGTKFSFTISSLQ PEDFATYYC FG Q GTKLE IK				LC-08
DIQMTQSPAS L ISASV GDRVTITC WY L QKPGKSPQ LIY GVPSRFSGSGSGTKFSFTISSLQ PEDFATYYC FG Q GTKLE IK				LC-09
DIQMTQSPAS L ISASV GDRVTITC WY L QKPGKSPQ LIY GVPSRFSGSGSGTKFSFTISSLQ PEDFANYYC FG Q GTKLE IK				LC-10
DIQMTQSPAS L ISASV GDRVTITC WY L QKPGKSPQ LIY GVPSRFSGSGSGTKFSFTISSLQ PEDFANYYC FG Q GTKLE IK				LC-11
DIQMTQSPAS L ISASV GDRVTITC WY L QKPG Q SPQ LIY GVPSRFSGSGSGTKFSFTISSLQ PEDFANYYC FG Q GTKLE IK				LC-12

FIG. 12A

Light Chain CDR Sequences of cH36

CDR1 (11 AA)	CDR2 (7 AA)	CDR3 (9 AA)
24 34 50 56 97		
L A S Q T I D T W L A A A T N L A D Q Q V Y S S P F T		

FIG. 12B

FIG. 12C

FIG. 12D

Sequences of Partially and Fully Humanized Heavy Chain (LC) Variable Regions

Heavy Chain (HC) FR Sequences

FR1 (30 AA)	FR2 (14 AA)	FR3 (32 AA)	FR4 (11 AA)	Names
1	29	67	95	
10	44	75	107	
20	36	67	95	
29	36	67	95	
36	67	75	107	
44	67	75	107	
50	67	75	107	
57	67	75	107	
64	67	75	107	
71	67	75	107	
78	67	75	107	
85	67	75	107	
92	67	75	107	
99	67	75	107	
106	67	75	107	
113	67	75	107	
120	67	75	107	
127	67	75	107	
134	67	75	107	
141	67	75	107	
148	67	75	107	
155	67	75	107	
162	67	75	107	
169	67	75	107	
176	67	75	107	
183	67	75	107	
190	67	75	107	
197	67	75	107	
204	67	75	107	
211	67	75	107	
218	67	75	107	
225	67	75	107	
232	67	75	107	
239	67	75	107	
246	67	75	107	
253	67	75	107	
260	67	75	107	
267	67	75	107	
274	67	75	107	
281	67	75	107	
288	67	75	107	
295	67	75	107	
302	67	75	107	
309	67	75	107	
316	67	75	107	
323	67	75	107	
330	67	75	107	
337	67	75	107	
344	67	75	107	
351	67	75	107	
358	67	75	107	
365	67	75	107	
372	67	75	107	
379	67	75	107	
386	67	75	107	
393	67	75	107	
400	67	75	107	
407	67	75	107	
414	67	75	107	
421	67	75	107	
428	67	75	107	
435	67	75	107	
442	67	75	107	
449	67	75	107	
456	67	75	107	
463	67	75	107	
470	67	75	107	
477	67	75	107	
484	67	75	107	
491	67	75	107	
498	67	75	107	
505	67	75	107	
512	67	75	107	
519	67	75	107	
526	67	75	107	
533	67	75	107	
540	67	75	107	
547	67	75	107	
554	67	75	107	
561	67	75	107	
568	67	75	107	
575	67	75	107	
582	67	75	107	
589	67	75	107	
596	67	75	107	
603	67	75	107	
610	67	75	107	
617	67	75	107	
624	67	75	107	
631	67	75	107	
638	67	75	107	
645	67	75	107	
652	67	75	107	
659	67	75	107	
666	67	75	107	
673	67	75	107	
680	67	75	107	
687	67	75	107	
694	67	75	107	
701	67	75	107	
708	67	75	107	
715	67	75	107	
722	67	75	107	
729	67	75	107	
736	67	75	107	
743	67	75	107	
750	67	75	107	
757	67	75	107	
764	67	75	107	
771	67	75	107	
778	67	75	107	
785	67	75	107	
792	67	75	107	
799	67	75	107	
806	67	75	107	
813	67	75	107	
820	67	75	107	
827	67	75	107	
834	67	75	107	
841	67	75	107	
848	67	75	107	
855	67	75	107	
862	67	75	107	
869	67	75	107	
876	67	75	107	
883	67	75	107	
890	67	75	107	
897	67	75	107	
904	67	75	107	
911	67	75	107	
918	67	75	107	
925	67	75	107	
932	67	75	107	
939	67	75	107	
946	67	75	107	
953	67	75	107	
960	67	75	107	
967	67	75	107	
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981	67	75	107	
988	67	75	107	
995	67	75	107	
1002	67	75	107	
1009	67	75	107	
1016	67	75	107	
1023	67	75	107	
1030	67	75	107	
1037	67	75	107	
1044	67	75	107	
1051	67	75	107	
1058	67	75	107	
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1240	67	75	107	
1247	67	75	107	
1254	67	75	107	
1261	67	75	107	
1268	67	75	107	
1275	67	75	107	
1282	67	75	107	
1289	67	75	107	
1296	67	75	107	
1303	67	75	107	
1310	67	75	107	
1317	67	75	107	
1324	67	75	107	
1331	67	75	107	
1338	67	75	107	
1345	67	75	107	
1352	67	75	107	
1359	67	75	107	
1366	67	75	107	
1373	67	75	107	
1380	67	75	107	
1387	67	75	107	
1394	67	75	107	
1401	67	75	107	
1408	67	75	107	
1415	67	75	107	
1422	67	75	107	
1429	67	75	107	
1436	67	75	107	
1443	67	75	107	
1450	67	75	107	
1457	67	75	107	
1464	67	75	107	
1471	67	75	107	
1478	67	75	107	
1485	67	75	107	
1492	67	75	107	
1499	67	75	107	
1506	67	75	107	
1513	67	75	107	
1520	67	75	107	
1527	67	75	107	
1534	67	75	107	
1541	67	75	107	
1548	67	75	107	
1555	67	75	107	
1562	67	75	107	
1569	67	75	107	
1576	67	75	107	
1583	67	75	107	
1590	67	75	107	
1597	67	75	107	
1604	67	75	107	
1611	67	75	107	
1618	67	75	107	
1625	67	75	107	
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1660	67	75	107	
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1688	67	75	107	
1695	67	75	107	
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1716	67	75	107	
1723	67	75	107	
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1737	67	75	107	
1744	67	75	107	
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1758	67	75	107	
1765	67	75	107	
1772	67	75	107	
1779	67	75	107	
1786	67	75	107	
1793	67	75	107	
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1814	67	75	107	
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1828	67	75	107	
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1975	67	75	107	
1982	67	75	107	
1989	67	75	107	
1996	67	75	107	
2003	67	75	107	
2010	67	75	107	
2017	67	75	107	
2024	67	75	107	
2031	67	75	107	
2038	67	75	107	
2045	67	75	107	
2052	67	75	107	
2059	67	75	107	
2066	67	75	107	
2073	67	75	107	
2080	67	75	107	
2087	67	75	107	
2094	67	75	107	
2101	67	75	107	
2108	67	75	107	
2115				

hOAT (IgG1) CONSTANT REGIONS SEQUENCES

SEQUENCES OF LC CONSTANT:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTLSSTLTLSKADYEKH

KVYACEVTHQGLSSPVTKSFNRGEC

FIG. 14A

SEQUENCES OF HC CONSTANT:

EFASTKGPSVFPPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVPSSSLGTQTYIC

NVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSSHEDPEVKFNWYVDGVEV

HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYITLPPSRDELTKNQVSLTCL

VKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK

FIG. 14B

hFAT (IgG1) CONSTANT REGION SEQUENCES

SEQUENCES OF LC CONSTANT:

RTVAAPSVFI FPPSDEQLKSGTASVVCLLNFFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSLTLSKADYEK

HKVYACEVTHQGLSSPVTKSFNRGEC

FIG. 15A

SEQUENCES OF HC CONSTANT:

EFASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVFPSSSLGTTY

TCNVDPKPSNTKVDKRVESKYGPPCPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGV

EVHNAKTPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIISKAKGQPREPQVYITLPPSQEEMTKNQVSL

TCLVKGFYPSDDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSMHEALHNHYTQKSLSLGLGK

FIG. 15B